Experimental Application Of The Balsam Fir Sawfly Nucleopolyhedrovirus Against Its Natural Host, The Balsam Fir Sawfly

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Nature of Proposed Pesticide Application

The Province of Newfoundland and Labrador continues to face serious and widespread infestations of balsam fir sawfly (*Neodiprion abietis* – Hymenoptera: Diprionidae). These infestations are threatening substantial investments in silviculture and consequently the long-term wood supply for the forest industry. For a fourth year, the Canadian Forest Service (CFS), in co-operation with the Newfoundland Department of Forest Resources and Agrifoods (DFRA) and Forest Protection Limited (FPL), is proposing to carry out an experimental research application of a highly species-specific microbial biological control agent (balsam fir sawfly nucleopolyhedrovirus – NeabNPV) on selected silviculturally treated forest stands forecast to receive moderate to severe the balsam fir sawfly defoliation in 2003 and at the leading edge of the infestation. Applications of this biological control agent will be made using fixed-wing aircraft and/or helicopters.

Description of Balsam Fir Sawfly Problem

Insect population levels

The balsam fir sawfly is native to and has been an occasional pest on balsam fir in Newfoundland. Recently, it has become more important as a pest of young and semi-mature balsam fir (*Abies balsamea*), particularly in pre-commercially thinned stands (PCTs). The population overwinters in the egg stage in fir needles and larvae usually hatch in late-June to mid-July depending on the weather. Larvae feed on previous-year and older foliage for a number of weeks before pupating. Adult sawflies emerge in August, mate and eggs are laid in the needles of the current year. Populations have been regulated by natural parasites and predators. Outbreaks have normally been of short duration (3 - 4 years) and were terminated by natural factors, predominantly NeabNPV. Although localized damage was often severe, tree mortality was limited. Defoliation, however, can cause significant growth loss to affected trees without tree death. Research at CFS has shown that, after defoliation has ceased, there may be a 13- to- 18-year period of reduced growth before the trees recover to pre-infestation growth rates (Piene et al. 2001).

The current infestation in western Newfoundland was detected in 1991 near Bottom Brook, east of Stephenville. By 1994, approximately 1,216 hectares (ha) of defoliation were recorded. In 1995, high population levels were observed. Moderate and severe defoliation was mapped on 12,600 ha, with some 10 percent mortality occurring in young fir stands. The situation in 1996 saw the infestation continue to expand with defoliation on 19,700 ha, including 15,400 ha in the moderate and severe categories. In 1997, the infestation expanded to the northeast and southeast into larger areas of valuable balsam fir (PCT) stands. A total of 53,000 ha were defoliated in 1997 with 30,300 ha in the moderate and severe categories. Pockets of defoliation were also detected on the Burin Peninsula and in Bay d'Espoir. The moderate and severe defoliation in 1998 totaled approximately 24,400 ha with 16,500 ha occurring in western Newfoundland, 5,800 ha in Bay d'Espoir and 2,100 ha on the Burin Peninsula. In 1999, moderate and severe defoliation occurred on 18,400 ha with 12,400 ha in western Newfoundland, 3,300 ha in Bay d'Espoir and 2,800 ha on the Burin Peninsula. In 2000, approximately 22,000 ha in western Newfoundland and 19,000 ha in the Bay d’Espoir were defoliated. Moderate to severe defoliation was recorded on 38,000 ha in western Newfoundland and 9,000 ha in the Bay d'Espoir in 2001.

In 2002, moderate to severe defoliation reached 60,000 ha in western and southern Newfoundland. The western area extended from south of Grand Lake, north to Old Man's Pond and from Stag Lake-Cook's Brook across the Humber Arm near Gillams and east to Steady Brook-Corner Brook Lake. This area is of particular concern because a significant proportion is PCT. These PCTs have been established, at an average cost of $1,000+/ha (a total amount in excess of $10 million). These are critical to maintaining an adequate wood supply for the forest industry.

The impact of balsam fir sawfly infestations, if left unchecked, will result in substantial loss of this investment. The failure to adequately protect the investment in silviculture, and the potential loss of future harvestable stands, would be significant to the social and economic well-being of the people, particularly on the west and south-west coasts of the Island. This is true both in terms of direct employment and in spin-off economics.

Apart from NeabNPV, there does not appear to be any other significant natural factor influencing balsam fir sawfly populations. With prolonged, severe defoliation, affected trees will be stressed, lose growth and be subject to mortality from secondary insects and diseases. It is estimated that, since the balsam fir sawfly outbreak began, the Province has lost is excess of 2 m^3 of growth per hectare per year, a loss in excess of 120,000 m^3 of incremental growth.

Control Options

A pest management program is being developed against the balsam fir sawfly in Newfoundland to protect valuable young stands and silviculturally treated areas of balsam fir. The purpose of the program is to reduce balsam fir sawfly population levels in treated areas to minimize the loss of foliage, tree growth and to prevent tree mortality due to secondary infestations in trees weakened by balsam fir sawfly attack. Unfortunately, control options for balsam fir sawfly are limited. Experimental programs were carried out by CFS and its collaborators in 1998, 1999, 2000, 2001 and 2002 in Newfoundland and in other jurisdictions to develop biological control options for a number of sawflies, including the balsam fir sawfly, yellowheaded spruce sawfly (*Pikonema alaskensis*), pine false webworm (*Acantholyda erythrocephala*) and introduced pine sawfly (*Diprion similis*). Progress has been made and work is continuing.

Dylox

The organophosphate insecticide, Dylox 420 (trichlorfon) is no longer considered as an option. It was used in 1998 under an emergency registration from PMRA. Based on experimental trials conducted in the same year, it was determined that lower dosages than those recommended could be effective. DFRA requested registration of Dylox and, for 1999 only, PMRA granted a temporary registration for Dylox for use against balsam fir sawfly. There were a number of conditions related to buffer zones (no spray zones), dose parameters and monitoring requirements attached to the temporary registration.

Dylox is fully registered for use against the yellowheaded spruce sawfly but is not being pursued further for balsam fir sawfly. This is due to public resistance to its use and issues surrounding buffer zones. In 1998, buffer zones for Dylox around water bodies were established at 100 m at the federal level and 200 m provincially. This restricted control measures to approximately 3,100 ha. In 1999, buffer zones were established at 200 m both federally and provincially. This resulted in the further exclusion of significant areas of infested stands from the protection program.

Bacillus thuringiensis
The most common biological insecticide to be applied aerially in forests against the spruce budworm and hemlock looper is *Bacillus thuringiensis* var. *kurstaki* (B.t.k.). B.t.k. was developed as a control product for, certain pest insects belonging to the order Lepidoptera (butterflies and moths). For B.t.k. to be effective, it must be ingested by an appropriate host insect. A protein crystal within the wall of the bacterial spore must first be digested by specific proteases within the alkaline midgut of the host insect. The B.t.k. toxin must bind to specific receptors on the midgut epithelial cells to work. Sawflies belong to the order Hymentoptera (includes bees, ants and wasps) and their larvae are not susceptible to B.t.k.

Bacillus thuringiensis var. *israelensis* (B.t.i.), registered for use in the control of mosquito and blackfly (order Diptera) larvae. Its mode of operation is the same as that of B.t.k. but proteins digested from the larger crystal bind specifically to receptors on the cells of the midgut of larval mosquitoes and blackflies not those of lepidopteran larvae. In 1999, B.t.i. was tested experimentally against balsam fir sawfly on a small area. B.t.i. was found not to be effective.

Neem
Neem (azadirachtin) is a botanical insecticide extracted from the neem tree (*Azadirachta indica*) native to India and parts of Africa. Certis (a manufacturer of one neem product) applied for and received temporary registration from PMRA for Neemix 4.5 for use against several sawfly species including the balsam fir sawflies. Neem has a number of properties that affect target pests. Depending on the amounts applied, these include insecticidal, insect growth regulatory and anti-feedant activities. Neem is registered for use in many countries including the USA where it is registered for indoor and outdoor use. It may be applied aerially and/or from the ground to horticultural-ornamental plants, trees, shrubs and agricultural crops. An operational program using Neemix 4.5 was carried out by DFRA on about 1,500 ha in the Bay d'Espoir in 2001. In 2002, Neemix 4.5 was applied to just over 6,000 ha near Corner Brook. Neemix 4.5 is not available for use in 2003 because the temporary registration has expired.

Balsam fir sawfly nucleopolyhedrovirus
Nucleopolyhedroviruses (NPVs) are a large group of viruses with covalently closed, double-stranded DNA genomes of 88-153 kb. NPVs are found only in arthropods, primarily insects. NPVs have a high degree of host specificity affecting a single insect species or only ones that are closely related. NPVs are not related to any known human, veterinary or plant pathogenic viruses. Specificity and safety tests of NPVs have shown that there are no toxicological or other deleterious effects on mammals, birds, amphibians, aquatic microorganisms and beneficial and other nontarget insects. Population crashes due to NPV epidemics occur in

many species of sawflies. NPVs are transmitted through ingestion by a suitable host larva. Viral polyhedrin inclusion bodies (PIBs) dissolve in the midgut, releasing the virions to infect midgut epithelial cells. Sawfly NPVs only infect the midgut epithelium so that, following a single replicative cycle, infected cells containing PIBs are sloughed off into the frass and out of the body where they can infect other host insects. Death normally occurs within 1 to 2 weeks but, during that time, the host is producing infective units of the disease. Sawfly NPVs are highly host specific and it has been necessary to develop a different virus for each host species. Attempts to use NPVs to suppress sawfly populations have usually met with success.

Progress 1997-2002

Field trials

In 1999, we applied NeabNPV to 1 ha of balsam fir forest in order to field amplify the virus. From this 1-ha application, we obtained enough NeabNPV to treat 1,800 ha of forest at an application rate of 1 x 10^9 PIBs/ha. On July 22-23, 2000, three blocks, each 50 ha in area, between Pinchgut Lake and Big Gull Pond near Corner Brook, Newfoundland, were treated aerially with NeabNPV at 3 x 10^9 PIBs/ha. (In all trials, a 20% aqueous solution of molasses was used as the carrier for the virus and the mixture was applied at a rate of 2.5 L/ha using Cessna 188 AgTrucks equipped with Micronair AU4000 atomizers. Aerial field trails (1 x 10^9 PIBs/ha) were conducted on July 21-22, 2001, east and north of Stag Lake near Corner Brook and north of St. Alban's, Bay D'Espoir, on July 24, 2001. The three blocks near Stag Lake totaled 2200 ha and the block in the Bay D'Espoir was 600 ha. On July 21-23, 2002, a total of approximately 5000 ha was treated (1 x 10^9 PIBs/ha) in three blocks to the south, east and north of Corner Brook. In all trials in all three years, there was good deposit on the targeted areas resulting in significantly higher larval mortalities in the spray blocks compared to the control blocks. Additionally, it was found that the number of balsam fir sawfly pupae and eggs was lower in the spray blocks compared to the control blocks in the year of the spray. In the year immediately following NeabNPV applications, we have found that the number of eggs per shoot, the percentage of successful egg hatch and the resultant number of larvae per shoot were lower in the spray blocks than in the controls. As a result, defoliation in the control blocks was much greater than in any of the spray blocks, which had little defoliation. An objective of the 2002 field trial was to determine if application of NeabNPV against first-instar balsam fir sawfly would kill the insects in time to give some foliage protection in the year of application. There was some foliage protection but it was limited.

In the field trials of NeabNPV to date, we have found that i) NeabNPV is easy and cheap to produce, ii) our formulation allows for smooth flow from the aircraft and good deposit on the foliage, iii) a single application at 1- 3 x 10^9 PIBs/ha against first- and second-instar larvae results in large reductions in the larval population within 15 days and iv) application in one year appears to affect the population of balsam fir sawfly larvae in the next year resulting in significantly decreased defoliation. In the three years of our field trials, we have not observed any adverse effects to the environment or to human health and, to our knowledge, none have been reported by any other party.

Field Trials in 2003

The balsam fir sawfly infestation was apparent in Corner Brook and on the north side of the Humber Arm (Summerside – Hughes Brook) in 2001. In 2002, the infestation had spread further towards the northeast and we sprayed the balsam fir sawfly infestation near Old Man's Pond with NeabNPV. On the other side of the Humber River, defoliation could be seen up the valley past Steady Brook towards Little Rapids. We propose to spray this leading edge of the population to see if we can delay or halt its advance.

NeabNPV will be applied against first- and second-instar balsam fir sawfly larvae at a rate of 1 x 10^9 PIBs/ha. A 20% aqueous solution of molasses will be used as the carrier for the virus and the mixture will be applied at a rate of 2.5 L/ha using Cessna 188 AgTrucks equipped with Micronair AU4000 atomizers. Experimental treatment blocks will have three transect lines, perpendicular to the line of aircraft flight, with 10 groups of three sampling trees spaced evenly along the length of the transect. Similar but untreated control blocks will also be established. In the treatment and control blocks, pre-spray samples will be collected shortly after 100% egg hatch has been reached and once a week beginning one week after the spray and for three subsequent weeks. Additionally, these blocks will be monitored in 2004 and 2005 to determine the impact of NeabNPV in years following NeabNPV application. Sprays will be monitored for product deposition and meteorological conditions using standard techniques. Defoliation estimates will also be made in August or September.

Separate spray blocks (approximately 1 ha each) will be established for NeabNPV production in areas where there is high balsam fir sawfly larval densities. These blocks will be sprayed at a rate of 3 x 10^9 PIBs/ha when the larval index is peak second instar. Infected larvae will be collected and NeabNPV purified using established methods.

CFS and its cooperators utilize appropriate current equipment and technology. CFS complies with existing regulatory guidelines. Navigation of spray aircraft is by a Differential Global Positioning System (DGPS) located inside the aircraft. Parameters of the spray blocks will be determined on the ground using hand-held GPS personal navigators, and these coordinates will be transferred to the on-board aircraft computer. At the time of the spray application a supervisor in an accompanying aircraft will observe the application from above to determine the accuracy and performance of the spray aircraft and will initiate corrective action as necessary. CFS supervisors will assesses the favourability of weather parameters before and during spray application. To ensure environmental safety, spray bases will have appropriate, current and approved safety and emergency equipment, materials and methods.

Worker Safety

CFS has well-established safety guidelines for workers involved in insect control. Personnel handling the NeabNPV formulation (mixer/loader) will wear the required safety equipment as indicated on the experimental label during mixing and loading onto the aircraft. In addition, approved safety precautions and established rules and guidelines will be adhered to concerning personal hygiene of all mixer/loader personnel working with NeabNPV formulations as indicated on the experimental label. Hospital and emergency telephone numbers will be posted in a conspicuous place to be used in the event of accident. Applicable contingency measures will be available to personnel in the event of an accident.

Public Health Considerations

To minimize the risk of exposure of people to insecticide spray, "no-spray" buffer zones will be left around known places of permanent human habitation and around areas such as cabin developments, parks, camps and day use areas. In 2003, spraying near habitation will be subject to terms and conditions of the Operator's Licence from the Department of Environment & Labour in consultation with the appropriate Health and Community Services personnel. Cabins will be adequately buffered in relation to the product being applied. In addition, a 1.6 km buffer zone is left around identifiable intakes to known community water supplies. If, during the course of a spray mission, unauthorized personnel are detected in or near a treatment area, the aerial supervisor will instruct the spray aircraft pilot to provide extra buffers or to terminate the mission, as circumstances dictate.

Environmental Safety

In terms of environmental safety, all stipulations in the licence issued by the provincial Department of Environment & Labour will be followed. These include the reporting of any incidents, such as spills, to the appropriate authorities. In connection with this, CFS and DFRA have contingency plans that are reviewed and approved annually prior to receiving of an Operator's Licence. These plans outline procedures for spill reporting, emergency first aid for exposure, insecticide spill only, aircraft crash in bush, aircraft accident on or near the airport, jettisoned aircraft load, drum decontamination and disposal and other general regulations and instructions as necessary.

Public Notification

As part of the program, the public and media in the vicinity of the proposed treatment areas will be notified, prior to commencement of the program, through advertisements and/or news releases and through appropriate direct contact if required. Information included will be the product being used, general areas of spray blocks, timing of application, contact numbers, etc. Access roads to the general areas will be posted with signs indicating treatment, product, dates, and phone numbers for more information. A phone-in information line will be set up and the general public can call to find out the status of areas receiving treatment.

Regional offices of DFRA and the Department of Environment and Labour, as applicable, will be provided with maps showing spray blocks. These maps will be made available for viewing by the general public during regular office hours. District offices of the DFRA will be made aware of spray blocks in their area and are provided with applicable detailed maps so they can inform the public on specific local blocks when requested. Also, Dr. Christopher Lucarotti (CFS-AFC), who is in charge of the efforts to get NeabNPV registered for operational use against the balsam fir sawfly, will be present in Newfoundland during the spray period and at least one week before and two weeks after the spray. He may be called upon at other times to assist in answering questions and concerns from the public.

Potential Spray Conflicts

There are always potential conflicts with insect control programs; for example, proximity to habitation, water supply areas, recreational areas (fishing, camping, berry picking) and potential impacts on wildlife. However, in approving a product at the federal registration level, and in granting a licence at the provincial level, mitigating measures are identified which eliminate or significantly reduce the potential for conflicts. These mitigating measures are outlined on the product label as approved by the PMRA and in terms of any buffer zones as stipulated in the Operator's Licence. In addition, the proponent is also required to post signs and advise the public about the program to lessen accidental exposure.

Integrated Pest Management Approach

In 1997, a cooperative research agreement involving the CFS, DFRA, Corner Brook Pulp and Paper Ltd. and Abitibi-Consolidated Inc. was initiated to investigate the ecology of the balsam fir sawfly. The prevalence of natural control factors such as viruses, fungi and parasites and their effect on balsam fir sawfly populations are being investigated. The impact of the balsam fir sawfly on and differences observed between thinned and unthinned stands is also being investigated. In 1998, additional financial resources were obtained through a Natural Sciences and Engineering Research Council (NSERC) – CFS – Industry grant which is administered through the University of New Brunswick. This funding continued through 2001. Funding for 2000-2003 was also obtained from the CFS Biotechnology Strategy, by CFS researchers, to study the functional genomics of NeabNPV. Additional funds have been obtained from the NSERC BioControl Network for the period 2001-2006. These cooperative research programs, in identifying natural factors that influence balsam fir sawfly populations, will hopefully lead to an integrated pest management strategy against this pest.

In November 2000, CFS research staff had a registration pre-submission consultation with officials from PMRA. The purpose of the consultation was to determine the requirements that would have to be met in order to get a registration for the operational use of NeabNPV. A great deal of progress has been made since then including: i) three years of field efficacy trials, ii) four years of field work by three graduate students studying balsam fir sawfly ecology, iii) the NeabNPV genome has been fully sequenced and iv) bioassays against non-target insects have been carried out. It is hoped that an application for NeabNPV registration will be submitted to PMRA not later than December 2003.

Approval Process

Any chemical manufacturer who wishes to sell a pesticide in Canada must first register that pesticide under the Pest Control Products Act. To receive registration, the manufacturer must follow the registration process administered by PMRA. Registration involves the submission of an application by the manufacturer. The company must first carry out extensive studies on the product. The application must be supported by a very thorough data package documenting the effects of the pesticide on users, bystanders and the environment

A scientific evaluation of the product is then performed by PMRA. The scientific evaluation may take years, as the evaluation may require long- and short-term human health effects, residues in food, ground water contamination, effects on wildlife and environmental fate. A registration will be granted only if the safety of the pesticide and its merit and value for the proposed use are found to be acceptable. If problems with the product are identified, registration will not be granted. All products are subject to reevaluation, with provision for suspension or cancellation.

Once the Federal Government approves a registration, the provincial governments become more involved. Each province has legislation dealing specifically with pesticide use in that province. In Newfoundland and Labrador pesticide use is regulated under the *Pesticides Control Act*. This legislation requires all organizations and companies using pesticides to apply for and receive a Pesticide Operator Licence. This licence regulates aspects of an operation not covered by federal legislation and requirements. As with federal regulations, the Pesticide Operator Licence is designed to minimize risk to human health and the environment. Aspects of a pesticide operation, such as buffer zones, spill response, public information and notification programs, monitoring requirements, weather conditions, are all specified in the license as they relate to a particular spray program. The federal registration system, combined with the provincial licensing and regulatory system, ensures that any pesticide that is used in Canada has passed a comprehensive environment/health evaluation.

Provincial legislation also requires individuals to be trained in the safe use of pesticides. Only individuals that successfully pass the provincial pesticide applicator exam (administered by the Department of Environment and Labour - Pesticides Control Section) are granted an applicator license and authorized to handle pesticides. Compliance and enforcement activities are also carried out by the Pesticides Control Section.

As with all commercial pesticide operations, the 2002 experimental NeabNPV program will be regulated by the Pesticides Control Section of the Newfoundland and Labrador Department of Environment and Labour.

Attachments

(1) Product Label.
(2) Maps of proposed NeabNPV application sites.
(3) Copy of Operators License Applicable to Forest Insecticide Use (Terms and Conditions) from the Newfoundland and Labrador Department of Environment and Labour.
(4) PMRA Research Permit.

4. Label

KEEP OUT OF REACH OF CHILDREN

GUARANTEE: *Neodiprion abietis Nucleopolyhedrovirus*, NeabNPV:
4×10^9 polyhedrin inclusion bodies (PIBs) per milliliter.

REGISTRATION NO: XXXXXX PEST CONTROL PRODUCTS ACT

Net Contents: 40 mL (1.6×10^{11} PIBs)

Canadian Forest Service – Atlantic Forestry Centre
P. O. Box 4000
Fredericton, New Brunswick, E3B 5P7

NOTICE TO USER: This control product is to be used only in accordance with the directions on this label. It is an offence under the *Pest Control Products Act* to use a control product act under unsafe conditions.

NATURE OF RESTRICTION: This product is to be used only in the manner authorized. Consult provincial pesticide regulatory authorities about use permits which may be required.

RESTRICTED USE

GENERAL INFORMATION: For use against balsam fir sawfly larvae in forests. Treat when larvae are actively feeding.

SHAKE WELL BEFORE USE

DIRECTIONS: Add contents to 400 L 20% aqueous solution of molasses. Spray foliage at a rate of 2.5L of mix/ha for 1×10^9 PIBs/ha. Provide a uniform deposit on foliage. Larvae must eat deposit of ABIETIVIRUS to be affected. Recommended droplet size is 100 µm.

PRECAUTIONS

KEEP OUT OF THE REACH OF CHILDREN

CAUTION – EYE IRRITANT

POTENTIAL SENSITIZER: May cause sensitization. Avoid contact with skin, eyes or clothing. Wear a long-sleeved shirt, long pants, waterproof gloves and eye goggles when handling, mixing/loading or applying the product and during all clean-up/repair activities. Wash thoroughly with soap and water after handling. Remove contaminated clothing and wash before reuse.

FIRST AID: In case of contact, flush skin or eyes with clean water. If irritation persists, obtain medical attention or contact a poison control centre. Take container, label or product name and PCP Registration Number with you when seeking medical attention.

DISPOSAL: Do not reuse container. Follow provincial instructions for any required cleaning of the container prior to its disposal. Make empty container unsuitable for use and dispose in accordance with provincial requirements. Return any unused, unwanted product to Canadian Forest Service.

4. Label (français)

ABIETIVIRUS

Insecticide Biologique à pulvériser

À USAGE RESTREINT pour la Foresterie

LIRE L'ÉTIQUETTE AVANT L'UTILISATION

GARDER HORS DE LA PORTÉE DES ENFANTS

GARANTIE: *Neodiprion abietis Nucleopolyhedrovirus*, NeabNPV:
4×10^9 corps d'inclusion polyhedriques (CIPs) par millilitre.

NUMÉRO D'HOMOLOGATION XXXXXX,
LOI SUR LES PRODUITS ANTIPARASITAIRES

Contenu net: 40 mL (1.6×10^{11} CIPs)

Service canadien des forêts– Centre de foresterie de l'atlantique
C.P. 4000
Frédéricton, Nouveau-Brunswick, E3B 5P7

AVIS À L'UTILISATEUR: Ce produit antiparasitaire doit être employé strictement selon le mode d'emploi qui figure sur la présente étiquette. L'emploi d'un tel produit dans des conditions dangereuses constitue une infraction à la *Loi sur les produits antiparasitaires*.
NATURE DE LA RESTRICTION: Ce produit doit être employé strictement selon le mode d'emploi autorisé. S'informer auprès des autorités provinciales pour vérifier si un permis d'utilisation est requis.

À USAGE RESTREINT

RENSEIGNEMENTS GÉNÉRAUX: Pour l'utilisation en forêt contre les larves du diprion du sapin. Appliquer lorsque les larves se nourissent activement.

BIEN AGITER AVANT L'EMPLOI

MODE D'EMPLOI: Ajouter le contenu à 400L d'une solution aqueuse de mélasse à 20%. Pulvériser sur le feuillage au taux de 2.5L de mélange/ha donnant 1×10^9 CIPs/ha. Appliquer uniformément sur le feuillage. Les larves doivent ingérer les gouttelettes de ABIETIVIRUS pour être affectés. La grosseur recommandée des gouttelettes est de100 μm.

PRÉCAUTIONS

GARDER HORS DE LA PORTÉE DES ENFANTS

ATTENTION-IRRITANT POUR LES YEUX

SENSIBILISANT POTENTIEL: Peut causer la sensibilisation. Éviter le contact avec les yeux, la peau ou les vêtements. Porter des vêtements longs, des gant imperméables et des lunettes de protection pour manipuler, mélanger/charger ou appliquer le produit et pendant les opérations de nettoyage et de réparations. Bien se laver à l'eau et au savon après avoir utilisé le produit. Retirer les vêtements contaminés et les laver avant de les porter à nouveau.

PREMIERS SOINS: En cas de contact avec la peau ou les yeux, rincer avec de l'eau propre. Si une irritation persiste, obtenir de l'aide médicale ou contacter un centre antipoisons. Apporter le contenant, l'étiquette ou prendre note du nom du produit et le numéro d'homologation LPA au moment d'obtenir de l'aide médicale.

ÉLIMINATION: Ne pas réutiliser le contenant. Se comformer à la réglementation provinciale pour le nettoyage requis du contenant avant la mise au rebut. Rendre le contenant inutilisable et l'éliminer conformément à la réglementation provinciale. Retourner tout produit inutilisé et dont on veut se départir au Service canadien des forêts.

Part 1.2 Product Profile and Proposed Use Patterns (from *Registration Guidelines for Microbial Pest Control Agents and Products*, Pro98-01).

i) *Neodiprion abietis Nucleopolyhedrovirus* NeabNPV Baculoviridae

ii) NeabNPV is a baculovirus within the genus *Nucleopolyhedrovirus* (NPV). NPVs are a large group of viruses with covalently closed, double-stranded DNA genomes of 88-153 kb (NeabNPV genome is approximately 95 kb). In NeabNPV, numerous virions are singly occluded within a polyhedrin inclusion body (PIB). Polyhedrin is a 29-kd protein. Baculoviruses are restricted to arthropods, mostly insects. NPVs have a high degree of host specificity affecting a single insect species or ones that are closely related. Sawfly NPVs are especially host specific and those described to date only seem to infect and replicate in the midgut epithelial cells of a single host species (Wallace and Cunningham 1995). Sawfly NPVs (including NeabNPV) are ingested by host larvae. Polyhedrin is dissolved in the gut releasing the virions. The virions fuse with the microvilli of the midgut epithelial cells and nucleocapsids are transported into the nucleus where they uncoat and undergo replication. Viral morphogenesis occurs in the nucleus and, eventually, the host cell dies and lyses releasing PIBs into the gut lumen of the host. PIBs pass out with the frass and are consumed by other host larvae. NeabNPV was isolated from balsam fir sawfly (*Neodiprion abietis*) larvae collected near Corner Brook, Newfoundland.

iii) The recommended application rate of sawfly NPVs is 1-5 x 10^9 PIBs/ha (see also Wallace and Cunningham 1995).

iv) Bioinsecticide (larvicide).

v) Domestic.

vi) Formulated in 20% aqueous molasses.

vii) Control of balsam fir sawfly (*Neodiprion abietis*)

viii) Site of application – balsam fir forest stands in western Newfoundland near Corner Brook.

ix) Not applicable

x) 1 x 10^9 PIBs/ha (up to 1 x 10^{10} PIBs/ha for the purpose of NeabNPV field production) suspended in 20% aqueous molasses and applied at a volume of 2.5L/ha.

xi) Single application to coincide with first and second larval instars, mid-June to early-July.

xii) Fix-winged aircraft or helicopter equipped with Micronair AU4000 nozzles.

xiii) Standard procedures for aerial applications (protective clothing, eyewear, etc.) to be followed.

xiv) Sawfly NPVs are only known to infect and replicate in their specific sawfly species hosts.

5.0 Data Requirements

5.1 Agent Specifications and Characteristics (***Part 2*** *of Registration Guidelines for Microbial Pest Control Agents and Products*, Pro98-01).

Part 2.0 Product Characterization and Analysis

Part 2.1 Natural Resources Canada
Canadian Forest Service – Atlantic Forestry Centre
1350 Regent Street
P.O. Box 4000
Fredericton, New Brunswick, E3B 5P7

Part 2.2 Natural Resources Canada
Canadian Forest Service – Atlantic Forestry Centre
1350 Regent Street
P.O. Box 4000
Fredericton, New Brunswick, E3B 5P7

Part 2.3 Natural Resources Canada
Canadian Forest Service – Atlantic Forestry Centre
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Part 2.4 ABIETIVIRUS

Part 2.5 *Neodiprion abietis Nucleopolyhedrovirus* NeabNPV Baculoviridae

Part 2.6 None.

Part 2.7 Characterization of the MPCA

Part 2.7.1 Origin, Derivation and Identification of MPCA

i) *Neodiprion abietis Nucleopolyhedrovirus* NeabNPV Baculoviridae
(Volkman et al. 1995).

ii) Balsam fir sawfly nuclear polyhedrosis virus (Olofsson 1972).

iii) NeabNPV.

iv) In August 1997, balsam fir sawfly larvae were collected from two plots near Corner Brook, Newfoundland. These insects were reared in our laboratory in Fredericton, New Brunswick and larvae that died in rearing were examined for the presence of NeabNPV. This virus was found in a number of larvae and was isolated. Virus amplification of NeabNPV was carried out at the Pasadena Field Station in July and August in 1998 and 1999. Here, larvae were reared on balsam fir foliage in 5-L plastic tubs. NeabNPV was applied to the foliage and dead insects were picked from the foliage, by hand, and were frozen.

v) Stock isolates of NeabNPV are held at either 4°C or -20°C.

vi) Not applicable.

Part 2.7.2 Biological Properties of the MPCA

i) NeabNPV has been reported in populations of balsam fir sawfly from Alberta, Saskatchewan, Manitoba, Ontario, Quebec, Ontario (see Olofsson 1972) and Newfoundland (here). Balsam fir sawfly feed on one-year old and older foliage of balsam fir (*Abietis balsamea*).

ii) Balsam fir sawfly, *Neodiprion abietis* (Hymenoptera: Diprionidae). Larval infection is *per os*. NeabNPV virions infect epithelial cells of the larval midgut. Viral replication only known to occur in the nucleus of midgut epithelial cells of larval balsam fir sawflies (Federici 1993).

iii) NeabNPV is know to only infect and replicate in the midgut cells of balsam fir sawfly larvae. NeabNPV may cause mortality in other sawfly larvae, specifically *Acantholyda erythrocephala* (pine false webworm, Pamphiliidae), *Diprion similis* (introduced pine sawfly, Diprionidae), *Gilpinia hercyniae* (European spruce sawfly, Diprionidae), *Pristiphora geniculata* (mountain ash sawfly, Tenthredinidae). NeabNPV does not appear to replicate in these other sawfly species.

iv) Sawfly NPVs only infect the midgut epithelium so that following a single replicative cycle, infected cells, containing PIBs, are sloughed off into the frass and out of the body where they can infect other host insects. Host death normally occurs within a one to two weeks but, during that time, the host produces infective units of the disease.

v) No plasmids or extra chromosomal DNA. NeabNPV is a naturally occurring pathogen of the balsam fir sawfly and was isolated from that host. NeabNPV genome is a covalently closed, double stranded DNA approximately 95 kb in size. This virus has not been subjected to any type of nucleic acid recombination.

vi) NeabNPV can only be produced *in vivo* in balsam fir sawfly larvae. Currently, there are no tissue culture systems available for the production of NeabNPV. Like most baculoviruses, NeabNPV is probably sensitive to UV radiation.

vii) No unusual characteristics, morphological, physiological, biochemical or otherwise.

viii) Experimental ground applications of NeabNPV were made against balsam fir sawfly in Ontario in the early 1970s (Olofsson 1972). Other sawfly NPVs have been used, successfully, in trials against pest sawflies (Wallace and Cunningham 1995). Registration for use to control sawfly pests have previously been sought, in Canada, for two sawfly NPVs: Sertifervirus (*Neodiprion sertifer Nucleopolyhedrovirus*, NeseNPV) for European spruce sawfly (*Neodiprion sertifer*) and Lecontvirus (*Neodiprion lecontei Nucleopolyhedrovirus*, NeleNPV) for redheaded pine sawfly (*Neodiprion lecontei*) (see Wallace and Cunningham 1995).

5.2 Human Health Safety Testing

Medium scale field trial forestry

Baculoviruses are known to only infect arthropods, mostly insects. They are not related to any known vertebrate or plant pathogenic viruses. Specificity and safety tests of baculoviruses have shown that there are no toxicological or other deleterious effects to mammals, birds, aquatic organisms and beneficial or other nontarget insects.

Literature on specificity and safety testing of baculoviruses up to 1985 reviewed by Gröner (1986) and to 1989 by Laird et al. (1990). Additional literature from 1990 to the present was sought using searches of Current Contents, Agricola and the Canadian Research Index. No reports were found attributing any adverse effects of baculoviruses on nontarget organisms.

Part 2.8 Manufacturing Methods and Quality Assurance *(Registration Guidelines for Microbial Pest Control Agents and Products, Pro98-01).*

a) Preservation and Maintenance of the Productive Strain

Balsam fir sawfly larvae infected with NeabNPV from the original collection site have been frozen and stored at -20°C. PIBs isolated from host larvae may also be stored in water at 4°C.

b) Manufacturing Processes

NeabNPV in 20% aqueous molasses (commercial grade) is applied to balsam fir trees infested with balsam fir sawfly larvae using fixed-wing aircraft, helicopters, motorized ground-sprayers and/or backpack sprayers to the equivalent concentration of up to 1 x 10^{10} PIBs/ha. Collections of larvae begin at the first sign of larval mortality (about 7 days after application) and continue for the next 10 days. Larvae are knocked onto tarpaulins placed under balsam fir trees by beating the tree branches with a 2-m length of pruning pole. Collected larvae are transferred to 50-lb brown paper bags so that the bags are one-third filled with larvae and fir needles. Three, 30-cm, branch tips cut from balsam fir trees are added as a source of food and an additional 2-3 mL of NeabNPV suspended in water (1 x 10^7 PIBs/mL) is misted onto the foliage. The bag tops are folded over and stapled shut. The larvae are left in the bags to die or finish their development at ambient laboratory temperatures (18-20°C). Following the death of the larvae, the branch tips are removed and the contents of three bags are placed into a single, clean 50-lb brown paper bag. These bags are stapled shut and are stored in the laboratory at ambient temperature (18-20°C). By this time the needles and dead larvae are quite dry. Dead larvae, from these bags, are picked out from the needles, by hand, and are placed into 50-mL centrifuge tubes and frozen at -20°C. NeabNPV from the dead larvae are purified using the method described below.

NeabNPV isolation protocol - large scale.

1. Thaw and re-hydrate NeabNPV-infected balsam fir larvae in water, overnight.
2. Homogenize larvae in a 1000 mL beaker using a hand held blender.
3. Dilute with water and add 1% SDS to a concentration of 0.3% (final volume approximately 10 times the volume of dead larvae).
4. Add magnetic stirrer bar and stir for 60 min.
5. Filter through plastic mesh, save filtrate (contains NeabNPV).
6. Resuspend solid debris in 0.3% SDS and stir as in step 4 for 5 min.
7. Filter again through plastic mesh and repeat until clear filtrate is obtained.
8. Filter virus suspension through 8 layers of cheesecloth.
9. Centrifuge for 15 min in a Sorvall RC 28S centrifuge and HS-4 rotor (approx. 2000 x g).
10. Discard supernatant and add more of the NeabNPV suspension to centrifuge tubes, repeat steps 9 and 10 until all the NeabNPV suspension has been used.
11. Resuspend NeabNPV PIB pellets in 0.3% SDS and vortex.
12. Repeat centrifugation and resuspensed until a clear supernatant is obtained.
13. Pool NeabNPV PIB pellets.
14. Resuspend pellet in 0.5M NaCl, centrifuge.
15. Resuspend pellet in a small volume of water.

NeabNPV suspensions are centrifuged and stored in water at 4°C to inhibit growth of contaminating bacteria. To further reduce unwanted bacterial propagation, NeabNPV suspensions are only added to the molasses solution in the field immediately prior to use.

PIBs are quantified by combining a known volume of an unknown concentration of NeabNPV PIBs with a known volume of a known concentration of latex beads. Latex beads and PIBs from several fields of view are counted under the 100X oil lens of a compound microscope. The concentration of PIBs is determined as a proportion of the number of latex beads counted.

5.3 Food and Feed Residue Studies

Not applicable.

5.4 Environmental Fate and Environmental Toxicology

IR I: NeabNPV was isolated from western Newfoundland in the same ecozone (Zone 5) where it will be used.

References

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